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Abstract

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A perithecium growing on the surface of an agar slant may discharge its ripe ascospores quite freely and these can be seen as a black powder on the inside of the tube; whereas, when the perithecium grows below the surface inside the medium, it is unable to eject the ascospores which remain in dark masses after the perithecium disintegrates. The ascospores are ejected through a protruding neck of a perithecium after acquiring a definite state of maturity. Spore shedding occurs after eight to seventeen days, depending

upon the crosses and on the type of reproductive medium used. In certain crosses, they may not be noticed by even about the 22nd day. For purposes of random ascospore analysis, these ejected ascospores are generally isolated without any regard to selection which may be operating at the time of differential discharge exhibited by perithecia. It may be pointed out that certain workers suggest the isolation of ejected ascospores at least eighteen days after perithecium formation, but it is probable that the risk of selection due to 'differential ripening' of asci connected with 'differential discharge' cannot be avoided, even as late as this particular time lag. Perithecia have been found which do not evacuate their full contents altogether by a certain fixed time and some of them appear to do it by degrees. There are others which retain a part of their contents till the time of their disintegration.

Before the method for random ascospore isolation can be described, it may be necessary to have a brief insight into a perithecium. It may also be necessary to explain the outline of the mechanism involved in the 'differential discharge' of ascospores in relationship, particularly, to 'differential maturity' among asci.

A perithecium generally contains a cluster of asci. The cluster exhibits a pattern of its own, as to the arrangement of asci within a perithecium. Some of the asci are situated nearer the perithecial neck than the others and some of them are relatively more involved, as to different degrees of overlapping and crowding within the main body of a perithecium, than the others.

It is often observable that during the course of perithecial ripening, asci show different degrees of maturity. Some asci mature earlier than the others. Even within an ascus, some member ascospores are found at different levels of maturity. Genotype constitution of an ascus, along with other developmental factors, may well be responsible for 'differential maturity'. It may be noted that not only the position of different mature asci varies as to their relative distance from the perithecial neck (the place of discharge) but also the mature asci are found quite indiscriminately attached along with a number of unmatured ones within a perithecium. There appears to be thus a sort of nonlocalization and nonaggregation among the maturing asci.

As a perithecium, on reaching a right stage of maturity, generally starts shedding its ripe ascospores through its protruding neck, it is quite conceivable that those asci near the neck would be more favorably placed as to the shedding of their ascospores than the ones which are distantly situated from the neck. This is why it is not uncommon to find a number of mature asci within a perithecium that has stopped discharging its spores. It may be that, due to their genotypic constitution, certain asci gain such a position within a perithecium that renders them incapable of participating in ejecting their spores, even having reached the right type of maturity. Any sample isolated from the ejected spores may, therefore, preclude any chances of inclusion of genotypes from such asci and this may eventually lead to biased results.

Method: The collection of ascospores is undertaken directly from the fully matured perithecia, instead of the ascospores which are already ejected. This is achieved by running a pilot cross and noting the approximate time for shedding of the ascospores. Fully ripened perithecia are then removed from a cross which is made a day or two later than the pilot one and where no shedding seems to have been involved. A perithecium is judged to be suitable when it sheds spores immediately when placed in a drop of sterile water. All ascospores are isolated irrespective of their color in a microscopic field. ---Department of Botany, University of Malaya, Kuala Lumpur, Malaya.